Kariko, K., Ni, H., Capodici, J., Lamphier, M., and Weissman, D. (2004). J. Biol. Chem. *279*, 12542–12550.

Kariko, K., Buckstein, M., Ni, H., and Weissman, D. (2005). Immunity *23*, this issue, 165–175.

Koski, G.K., Kariko, K., Xu, S., Weissman, D., Cohen, P.A., and Czerniecki, B.J. (2004). J. Immunol. *172*, 3989–3993.

Sugiyama, T., Gursel, M., Takeshita, F., Coban, C., Conover, J., Kaisho, T., Akira, S., Klinman, D.M., and Ishii, K.J. (2005). J. Immunol. *174*, 2273–2279.

Tokunaga, T., Yamamoto, H., Shimada, S., Abe, H., Fukuda, T., Fujisawa, Y., Furutani, Y., Yano, O., Kataoka, T., and Sudo, T. (1984). J. Natl. Cancer Inst. *72*, 955–962.

Immunity, Vol. 23, August, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.immuni.2005.08.004

Central Tolerance Matters

In this issue of *Immunity*, Anderson et al. provide another clue to the riddle that is Aire—why do human beings and mice lacking Aire develop diffuse and pathogenic autoimmunity? They find that Aire influences central tolerance not only by promoting the expression of peripheral self-proteins in thymic medullary epithelial cells (MECs) but also by furnishing these cells with the apparatus for effective antigen presentation (Anderson et al., 2005).

In human beings, mutations in *Aire* lead to an autosomal recessive autoimmune disease termed APECED (autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy) or APS-1 (autoimmune polyglandular syndrome type 1) (Nagamine et al., 1997). Similarly, mice with a targeted disruption in *Aire* develop multiorgan inflammatory infiltrates and autoantibodies (Ramsey et al., 2002; Anderson et al., 2002). Analysis of *Aire*-deficient mice revealed that the inhibition of autoimmune disease requires MEC expression of *Aire*, and *Aire*-negative MECs have diminished expression of genes encoding otherwise tissue-restricted proteins (Anderson et al., 2002; Derbinski et al., 2005). A key to Aire's function may be revealed by its structure. *Aire* encodes a modular protein with two plant homeodomain (PHD)-zinc finger domains, a SAND domain common to several transcription factors (Gibson et al., 1998), a bipartite nuclear localization signal, and four nuclear-receptor binding motifs (Ramsey et al., 2002). Its expression is regulated by polyubiquitination and proteosomal degradation, and it associates with CBP and p300. Aire has all the characteristics of a transcriptional regulator, and yet it is a mystery—how does a transcriptional regulator promote the activation of a large and diverse group of tissue-specific genes united only as targets of autoimmunity? As a further complication, evidence has been presented suggesting Aire possesses E3 ubiquitin ligase activity mediated by one of its PHD homeodomains, and mutations affecting this enzymatic activity result in autoimmune disease (Uchida et al., 2004). This result highlights the potential for an additional function in maintaining self-tolerance.

How Does Aire Promote Self-Tolerance?

The expression of so-called "tissue-specific" proteins is observed in both human and mouse MECs (Gotter and Kyewski, 2004). One hypothesis posits this may provide a way for developing thymocytes to anticipate the variety of self-proteins potentially encountered while patrolling the body for invading pathogens (Gotter and Kyewski, 2004). Two mechanistic models have been proposed. In one scenario, negative selection of developing thymocytes would purge the repertoire of T cells reactive with Aire-induced self-proteins. A second possibility suggests self-reactive T cells may not be eliminated but rather promoted to the rank of regulatory T cells (T_{reg}). To distinguish these two possibilities, Anderson et al. evaluated the formation and function of T_{reg} and the negative selection of developing thymocytes in *Aire*-deficient mice. Although earlier reports did not observe any defect in the numbers of $CD4^+CD25^+$ T cells (Anderson et al., 2002; Liston et al., 2003, 2004), the functional T_{reg} activity of this population in *Aire*-deficient mice remained untested until recently (Anderson et al., 2005; Kuroda et al., 2005). *Aire*-deficient $CD4^+CD25^+$ T_{reg} possess wild-type (wt) levels of Foxp3 and effectively inhibit the proliferation of $CD4^+CD25^-$ T cells in vitro, indicating no defect in the regulatory lineage. Anderson et al. extended these studies to test regulatory function in vivo as well. In a well-characterized assay of autoimmune colitis induced by the transfer of purified $CD4^+CD25^-$ cells into lymphopenic mice, *Aire*-deficient and *Aire*-expressing $CD4^+CD25^+$ T_{reg} equally inhibited pathology and wasting (Anderson et al., 2005).

To test the possibility that other regulatory populations not possessing the $CD4^+CD25^+$ phenotype depend upon Aire expression, a second in vivo experimental strategy was employed (Anderson et al., 2005; Kuroda et al., 2005). Thymic stroma, from *Aire*-negative and -positive donors, were grafted into mice lacking their own thymus. In this case, the host T cell precursors populated both of the grafted thymic lobes and were selected by the criteria of the respective donor thymii. When wt and *Aire*-deficient thymic lobes were grafted at a 1:1 ratio, no improvement in the autoimmune infiltrates was observed (Anderson et al., 2005; Kuroda et al., 2005). The T_{reg} selected in the *Aire*-positive thymus could not restrain the autoimmune attack of the cells selected in the *Aire*-deficient thymus. Although a minor defect in T_{reg} has not been ruled out, together these data support the conclusion that Aire does not prevent autoimmunity by the positive selection of T_{reg}.

Defective Negative Selection in Aire-Deficient Mice

The role of Aire in negative selection of autoreactive thymocytes has been investigated by assessing the development of T cells expressing transgenic T cell antigen receptors of a single specificity in conjunction with a model tissue-specific antigen (Anderson et al., 2005; Liston et al., 2003, 2004). Liston et al. found that thymocytes expressing a receptor specific for hen egg lysozyme (HEL) are normally deleted in the presence of HEL expressed under control of either the rat insulin promoter (RIP) or thyroglobulin promoter; however, they failed to be deleted in the absence of Aire (Liston et al., 2003, 2004). In these mice, the expression of HEL driven by the tissue-specific promoters was, as predicted, significantly lower in the *Aire*-deficient MECs. Thus, efficient negative selection was impaired, resulting in the maturation of autoreactive thymocytes, peripheral autoreactivity, and diabetes (Liston et al., 2004). The same thymocytes were deleted properly when HEL was expressed under control of a systemic MHC class I promoter even in the absence of Aire. Anderson et al. similarly found the expected normal T cell deletion in two additional models with abundant expression of the model antigen (Anderson et al., 2005). In keeping with the proposed role of Aire in extending the expression of tissue-specific genes, Aire was also found to be required for deletion of OVA-specific MHC class I or class II-specific thymocytes and the prevention of diabetes when the transgenic antigen was expressed under the control of the RIP.

A Fresh Function for Aire: Antigen Presentation

As is often the case, the most important result appeared in the experimental controls. Although the expectation was that negative selection failed because of reduced RIP-OVA transcripts, this was not the case. The transcript levels for OVA were virtually identical in *Aire*-positive and *Aire*-negative MECs. Nonetheless, the deletion of thymocytes recognizing OVA was defective in the *Aire*-deficient thymus, suggesting a further, perhaps even more important function for Aire. This finding follows a recent study in which Aire-deficient mice were shown to develop an autoimmune infiltration associated with reactivity against a ubiquitous self-protein, α-fodrin, reminiscent of Sjögren's syndrome (Kuroda et al., 2005). In this case, the expression of α-fodrin transcripts in MECs was similarly unaffected by the loss of Aire. To find a solution to this enigma, Anderson et al. tested the ability of *Aire*-deficient MECs to present antigen to T cells. RIP-OVA transgenic MECs were unable to induce wt levels of proliferation when incubated with OVA reactive T cells, despite equal expression of OVA transcripts by both cell types. Similarly, *Aire*-deficient MECs pulsed with antigen did not stimulate proliferation as efficiently as their *Aire*-expressing counterparts, suggesting that the defect is not in antigen processing. So, what is amiss? Differences in the usual suspects of antigen presentation would have made for a satisfying conclusion to an engaging tale, but that was not to be. The levels of MHC and costimulatory molecules were unchanged in the *Aire*-negative MECs. Instead, microarray data intriguingly point to differences in the MEC expression of chemokines. Perhaps, these and other noted differences will lead to a new understanding of antigen presentation regulating negative selection.

Aire-deficient mice have been invaluable in highlighting the importance of MEC expression of otherwise tissue-specific proteins in promoting self-tolerance—once and for all time establishing the physiological importance of central tolerance in the avoidance of *horror autoxicus*. Furthermore, amidst the flurry of excitement surrounding the discovery of T_{reg} cells, these studies demonstrate that "recessive" negative selection is an essential aspect of self versus nonself discrimination. How can we know so much but understand so little? What are the aspects of antigen presentation missing in Aire-deficient MECs? How does a transcriptional regulator act on a widely diverse and seemingly unrelated set of genes? And finally, if Aire functions as an E3 ligase, what are its targets, and how does their turnover affect negative selection? The riddle of Aire has yet to be fully solved.

Ananda W. Goldrath and Stephen M. Hedrick
University of California, San Diego
La Jolla, CA 92093-0377

Selected Reading

Anderson, M.S., Venanzi, E.S., Klein, L., Chen, Z., Berzins, S.P., Turley, S.J., von Boehmer, H., Bronson, R., Dierich, A., Benoist, C., and Mathis, D. (2002). Science *298*, 1395–1401.

Anderson, M.S., Venanzi, E.S., Chen, Z., Berzins, S.P., Benoist, C., and Mathis, D. (2005). Immunity *23*, this issue, 227–239.

Derbinski, J., Gabler, J., Brors, B., Tierling, S., Jonnakuty, S., Hergenhahn, M., Peltonen, L., Walter, J., and Kyewski, B. (2005). J. Exp. Med. *202*, 33–45.

Gibson, T.J., Ramu, C., Gemund, C., and Aasland, R. (1998). Trends Biochem. Sci. *23*, 242–244.

Gotter, J., and Kyewski, B. (2004). Curr. Opin. Immunol. *16*, 741–745.

Kuroda, N., Mitani, T., Takeda, N., Ishimaru, N., Arakaki, R., Hayashi, Y., Bando, Y., Izumi, K., Takahashi, T., Nomura, T., et al. (2005). J. Immunol. *174*, 1862–1870.

Liston, A., Lesage, S., Wilson, J., Peltonen, L., and Goodnow, C.C. (2003). Nat. Immunol. *4*, 350–354.

Liston, A., Gray, D.H., Lesage, S., Fletcher, A.L., Wilson, J., Webster, K.E., Scott, H.S., Boyd, R.L., Peltonen, L., and Goodnow, C.C. (2004). J. Exp. Med. *200*, 1015–1026.

Nagamine, K., Peterson, P., Scott, H.S., Kudoh, J., Minoshima, S., Heino, M., Krohn, K.J., Lalioti, M.D., Mullis, P.E., Antonarakis, S.E., et al. (1997). Nat. Genet. *17*, 393–398.

Ramsey, C., Winqvist, O., Puhakka, L., Halonen, M., Moro, A., Kampe, O., Eskelin, P., Pelto-Huikko, M., and Peltonen, L. (2002). Hum. Mol. Genet. *11*, 397–409.

Uchida, D., Hatakeyama, S., Matsushima, A., Han, H., Ishido, S., Hotta, H., Kudoh, J., Shimizu, N., Doucas, V., Nakayama, K.I., et al. (2004). J. Exp. Med. *199*, 167–172.